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Review

Chromatographic analysis of local anesthetics in biological samples

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ABSTRACT

This paper reviews gas and liquid chromatographic methods for screening, identification and quantification of local anesthetics and/or their metabolites in biological samples. Basic information about sample preparation, separation, detection, and quantification of each procedure is summarised. The pros and cons of the various procedures are critically discussed.

Contents

1. Introduction

Local anesthetics are amino-amide, amino-ester and amino-ether compounds. They are used intravenously, epidurally, topically or subcutaneously to relieve pain during and after medical procedures. Absorption and distribution depend on many factors, such as site and method of administration, blood flow characteristics, plasma protein binding, plasma pH, the physical properties (i.e. p*Ka*, hydrophobicity, etc.) and chemical structure of the particular local anesthetic. The amino-ester local anesthetics are easily hydrolysed by the action of pseudocholinesterase in blood plasma, and are also rapidly decomposed in alkaline solutions non-enzymatically; amino-amide drugs are mainly metabolised by the liver microsomes and relatively stable in alkaline solution [1].

Also, many drugs that may affect the metabolism or pharmacokinetics of the anesthetics, such as opioids, cimeti-

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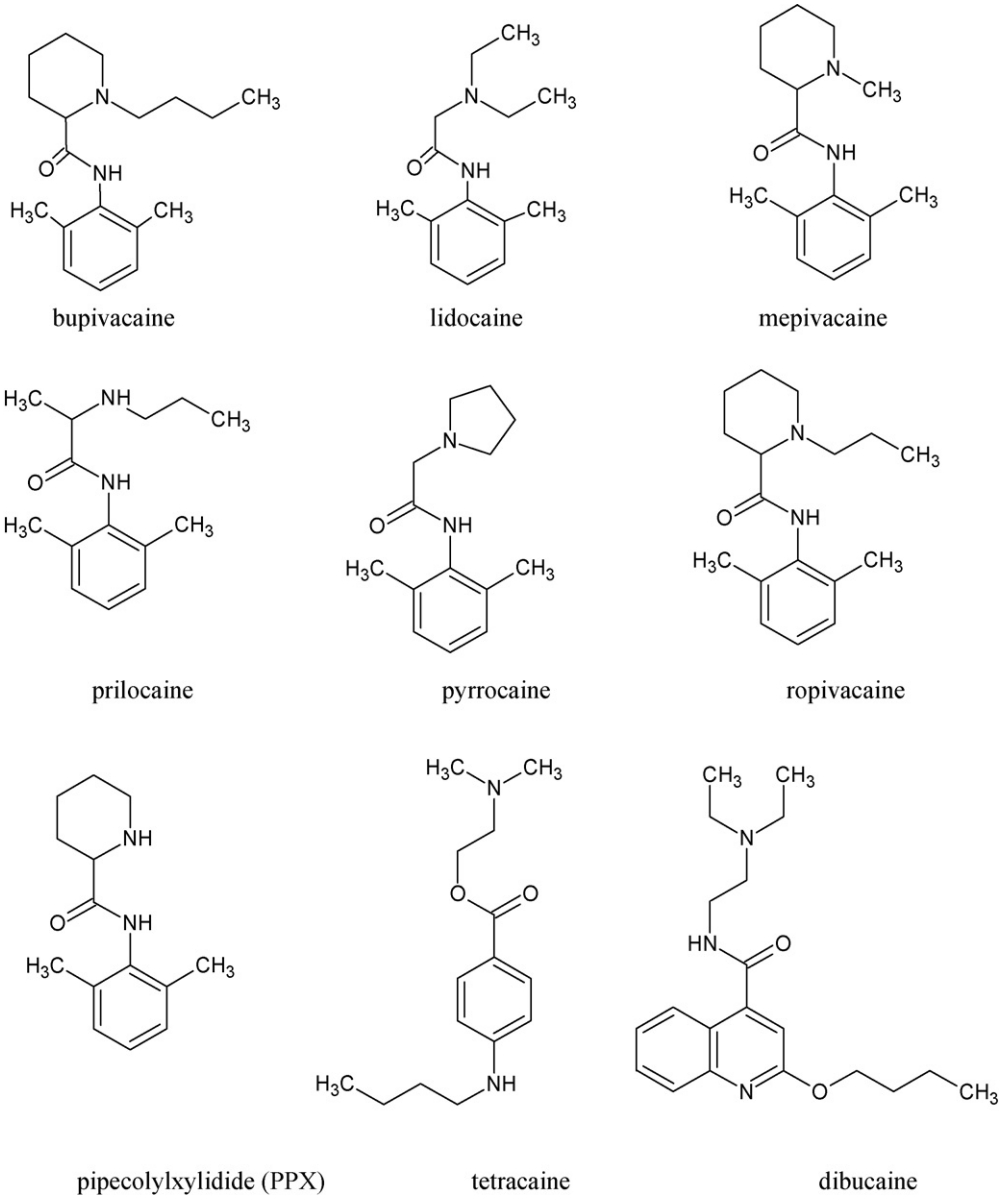


Fig. 1. Chemical structures of local anesthetics and some of their metabolites.

dine, or propanolol, are routinely administered during medical treatment.

Local anesthetics are potentially toxic if they are administered in high doses or into the wrong anatomic site, reaching the major target organs of toxicity, the brain and heart [2]. Toxic effects are seen at plasma levels of 5–10 μg/ml for lidocaine, mepivacaine and prilocaine and 1.5–4 μg/ml for bupivacaine, etidocaine, and ropivacaine [2]. The maximum tolerated doses for the above local anesthetics, excluding prilocaine, range from 1.4 to 9.8 mg/kg [2].

Determination of local anesthetics from biological samples is necessary to evaluate the pharmacokinetics and the metabolism of drugs, to establish appropriate dosage schemes in order to minimise adverse effects, and in analytical toxicology.

The anesthetics have been analysed by gas chromatography [3–13], liquid chromatography [14–48] and capillary electrophoresis [49–51].

This paper reviews GC and LC methods for screening, identification and quantification of local anesthetics and their metabolites in blood, plasma, serum, urine, and tissue. In Fig. 1 the chemical structures of some important local anesthetics and their metabolites are presented. In Tables 1 and 2 brief information about sample preparation, stationary phases/liquid phases, detection and quantification data of each procedure is summarised. Sample preparation, GC and LC separation, detection and application to pharmacokinetic studies are discussed more in depth below.

2. Sample preparation

Sample preparation is an important step in the development of GC and LC methods that aims to isolate the analytes of interest and to improve the selectivity, detectability, reliability, accuracy and repeatability of the analysis. It usually takes 75% of the total analysis time.

The most commune sample preparation methods used for the extraction of local anesthetics from biological samples are liquid–liquid extraction (LLE) and solid-phase extraction (SPE).

Recent trends in sample preparation have focused on the development of methods to reduce the sample volume required, the analytical time and the solvent consumption. Another trend con-

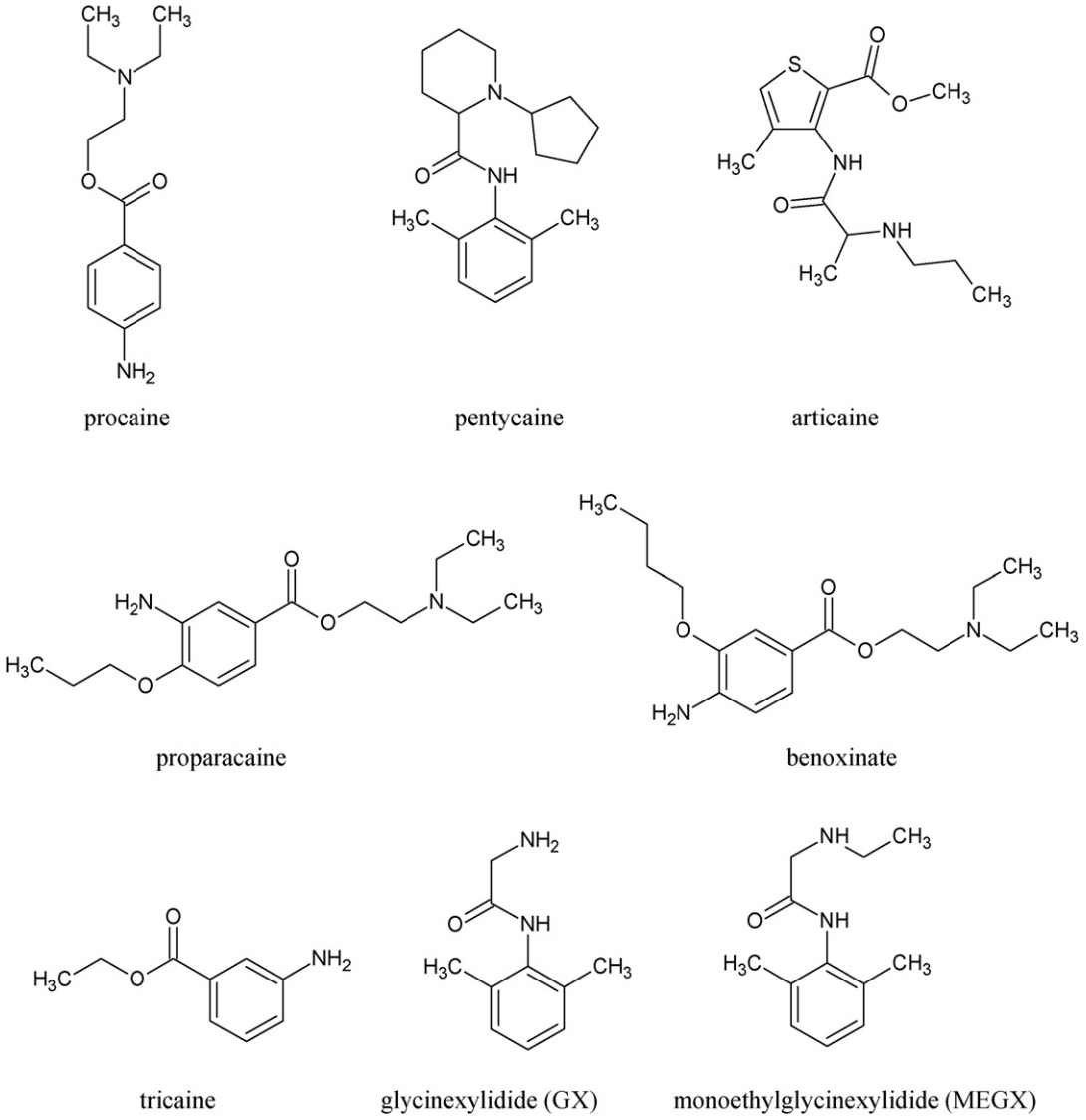


Fig. 1. (*Continued.*)

sists in the automation of extraction procedure by on-line coupling with analytical instruments. The recent sample preparation techniques include solid-phase microextraction (SPME), liquid–liquid microextraction (LLME), microextraction by packed sorbent (MEPS) and membrane extraction.

2.1. Liquid–liquid extraction

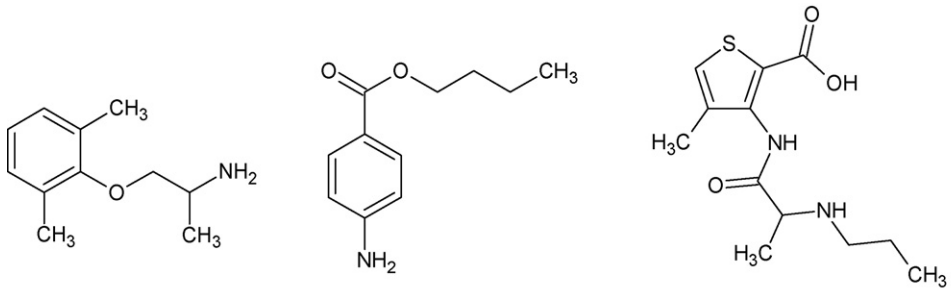
LLE is a frequently used method for extraction of local anesthetics from biological samples. The solvents utilised must have low solubility in water, low toxicity, and moderate volatility to facilitate the removal by evaporation, but not so volatile as to evaporate during sample preparation. As solvents *n*-hexane, *n*-heptane: methylene chloride (4:1, v/v), diethyl ether, cyclohexane, *t*-methylbutyl ether and *t*-ethylbutyl ether were used. Extraction of some local anesthetics from plasma and urine in methanol [20], from human blood in acetonitrile [42] or from plasma and urine in 2-propanol [32] is less likely or unlikely to realise due to the high solubility of these solvents in water. Practically, in these cases the organic layer doesn't separate from the aqueous layer.

For the efficient extraction of a base into an organic medium from an aqueous medium the pK_a of the aqueous medium should be at least 1 pH unit higher than the pK_a value of the base.

Extraction of ropivacaine from plasma and urine [8], of prilocaine [13], articaine [31] and bupivacaine [37] from human plasma, and proparacaine [28] from human aqueous humour has been performed following their alkalinisation with Na_2CO_3 or NaOH solutions. Bupivacaine, mepivacaine, ropivacaine and tetracaine (IS) were extracted from serum in *t*-butylethyl ether without serum alkalinisation [25]. At serum pH of 7.4 a large quantity of basic drugs remain in the aqueous phase (about 67% of mepivacaine remains as ionised fraction). A better separation of bupivacaine and lidocaine from endogenous acidic compounds from plasma was obtained by a back-extraction method [4,34].

Since the ester-type local anesthetics are rapidly hydrolysed by cholinesterase in blood it is necessary to use cholinesterase inhibitors, such as sodium fluoride, neostigmine bromide [1,9] or neostigmine methylsulfate [47]. Therefore, the introduction in the analytical vial of inhibitor before the sample is recommended [1].

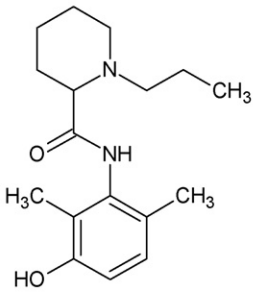
Extrelut (a diatomaceous earth) is a porous material that acts as a support for the aqueous phase. Extraction mechanism is similar to LLE. The sample (plasma and urine) was pH adjusted with NaOH and then applied to the Extrelut column. The elution



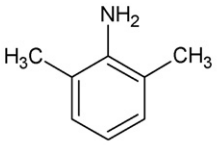
mexiletine

n-butyl-4-amino- benzoate

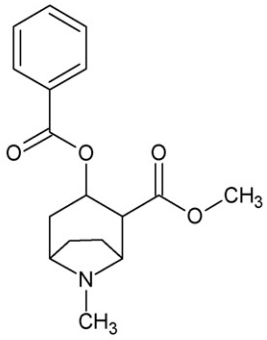
articainic acid



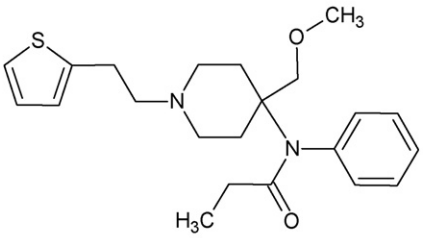
3-hydroxy-ropivacaine



2,6-xylidine



cocaine



sufentanil

Fig. 1. (*Continued.*)

of the analytes (prilocaine, lidocaine, mepivacaine, bupivacaine, MEGX, tetracaine, procaine, cocaine, dibucaine) was performed with dichloromethane-2-propanol solution (85:15, v/v) [9].

Liquid–liquid microextraction (LPME) is based on suspension of a single droplet of organic solvent, from the end of a microsyringe needle in an aqueous solution. The droplet containing analytes extracted by diffusion is injected directly into the GC or LC system. The optimal LPME conditions for extraction of some local anesthetics (lidocaine, bupivacaine and tetracaine) from human urine were: 6 ml of the feed solution (pH 11), 1 μl o-dibutylphthalate as the organic phase, 160 rpm of the steering rate and 30 min of the extraction time [27]. Nevertheless, this method is not quite robust since the droplets may be lost during the extraction.

2.2. Solid-phase extraction

SPE is used to extract and concentrate analytes from the liquid matrix by partitioning the compounds between a solid and a liquid phase. Reversed-phase (C8-, C18-silica), normal phase (cyanopropyl silica), ion – exchange sorbents and molecularly imprinted polymers (MIPs) and recently developed monolith silica sorbent, were used for extraction of local anesthetics from biological samples (e.g. plasma, serum, urine). The most frequently used sorbent is C18-silica [4,16,33,38]. At acidic or neutral pH local anesthetics exist largely in their ionised form, in which they are poorly retained by this lipophilic sorbent. Consequently, to secure a more complete retention the sample must be adjusted to alkaline pH. The removal of the activation solvent by a liquid similar in composition to the sample matrix is also necessary. Conditioning extraction cartridges with buffer solution pH 4.5, before sample introduction [7,38] or with water [16] determines ionisation of the analytes and their weak retention on the hydrophobic sorbent. Since analyte-phase interactions are hydrophobic (van der Waals forces) the analytes should be eluted with a less polar organic solvent that disrupts these forces. The washing of the cartridge with acetonitrile [16] determines the elution of analytes before the extraction itself.

Other types of sorbents for SPE extraction consist of porous polymers such as: styrene-divinylbenzene [39], acrylnitrile-

divinylbenzene [11] and cross-linked polystyrene [12]. The porous polymers are much more lipophilic than surface-modified silica, have a higher sample loading capacity and are stable at alkaline pH. For the analysis of mepivacaine, bupivacaine, ropivacaine and lidocaine (IS) from human serum [12], after sample alkalinisation, the extraction is briefly presented: following the conditioning with acetonitrile and methanol the adsorbent was brought to sample pH by its washing with buffer solution pH 10; after sample loading the cartridge was washed with buffer solution, pH 10 and the analytes were eluted with acetonitrile.

Included in the same category of sorbents, but with the unique ability to retain both non-polar and polar compounds is Oasis HLB. This sorbent is a macroporous copolymer made from a balanced ratio of two monomers, the liphophilic divinylbenzene and the hydrophilic N-vinylpyrrolidone. Due to this structure, acidic, basic and neutral compounds, polar and non-polar can be extracted by simply manipulating the organic solvent concentration and pH. The screening of 19 drugs including two local anesthetics (mepivacaine and lidocaine) from racehorse plasma was performed using on-line SPE coupled with LC-MS/MS [48]. The plasma was deproteinised (before SPE) with acetonitrile and acetic acid. After centrifugation, the supernatant was transferred in a Chrompack autosampler.

A new type of adsorbent, monolithic silica support is a silica-gel polymer with a bimodal pore structure (macropores and mesopores). A silica monolith is used after modification with functional group: octadecyl, phenyl, octyl and amino. In this support surface area per unit volume is large in comparison with particle-based silica support. Each step in SPE (conditioning, sample loading, washing, and elution of target compounds) is followed by centrifugation. The advantages of monolithic spin column extraction consist in: the flow rate may be easily controlled by monitoring the rotation speed and time; use of low sample volumes and low volumes of elution solvent. Monolith spin extraction was applied for determination of dibucaine and naphazoline in human serum [45]. Extraction recovery was not satisfactory for both drugs (within the range 70–78%).

Microextraction in a packed syringe technique (MEPS) is a new technique for miniaturised SPE that can be connected on-line to GC or LC. The main advantage of this method is the utilisation of small volume samples (10 μl), as well as large volumes. In MEPS any sorbent can be used as packing bed: C2, C8, C18, MIPs and ion-exchange sorbents are inserted into a syringe. Benzenesulphonic acid cation-exchange silica based sorbent, was utilised for the extraction of ropivacaine and its metabolites from human plasma [21]. When this mode of extraction is used, pH control during the loading, washing, and elution steps is important. During loading on a cation-exchange adsorbent the pH should be two units lower than the p*Ka* of the compound and two units higher than p*Ka* of the adsorbent. During elution the pH is chosen so that the functional groups of the compound and/or adsorbent are not charged, resulting in disruption of the ionic interactions.

Molecularly imprinted polymers (MIPs) constitute a novel class of sorbent of high selectivity. For ropivacaine and bupivacaine the extraction sorbent was methacrylic acid-ethylene glycol dimethacrylate prepared using pentycaine as the template [19]. An important disadvantage of this method is the leakage of template that affects accuracy and precision of analysis.

2.3. Solid-phase microextraction

This method is a solvent-free sample preparation technique often utilised for chromatographic analysis by which the analytes are extracted from a gaseous (headspace SPME) [10] or liquid sample (non-headspace SPME) [5,6].

Fibres coated with polydimethylsiloxane, carbowax/divinylbenzene polyacrylate and polydimethylsiloxane/divinylbenzene

Table 1
GC procedures used for analysis of local anesthetics.

Compound	Matrix	Extraction method	Stationary phase	Detection mode	Quantitative analysis	Reference
n-butyl-p-amino-benzoate; lidocaine; mepivacaine (IS)	Dog plasma	SPE	Trifluoro propylmethyl polysiloxane	NPD	Internal standard	[3]
Bupivacaine; pentycaine (IS)	Horse and human plasma	LLE	Dimethyl polysiloxane;	MS	Internal standard	[4]
Lidocaine	Spiked human urine	SPME	Dimethyl polysiloxane;	FID	External standard	[5]
Lidocaine	Human plasma	SPME	5%-phenyldimethyl polysiloxane	FID	External standard	[6]
Lidocaine; MEGX; mepivacaine (IS)	Human plasma	SPE	5%-phenyldimethyl polysiloxane	NPD	Internal standard	[7]
Ropivacaine; $^{2}H_{7}$-ropivacaine (IS); pentycaine (IS)	Plasma, urine, rat tissues	LLE	5%-phenyldimethyl polysiloxane	NPD, MS	Internal standard	[8]
Mepivacaine; propitocaine; lidocaine; procaine; cocaine; tetracaine; dibucaine; MEGX; bupivacaine (IS)	Human plasma and urine	SPE	Dimethyl polysiloxane	MS	Internal standard and external standard	[9]
Lidocaine; mepivacaine; bupivacaine; prilocaine; dibucaine; d_{10}-lidocaine (IS)	Human blood	SPME	Dimethyl polysiloxane	MS	Internal standard	[10]
Mepivacaine; lidocaine (IS)	Human serum	SPE	Polyetileneglycol	FID	Internal standard	[11]
Mepivacaine; lidocaine (IS); bupivacaine; ropivacaine	Human serum	SPE	Polyetileneglycol	FID	Internal standard	[12]
Prilocaine; lidocaine (IS)	Human plasma	LLE	5%-phenyldimethyl polysiloxane	NPD	Internal standard	[13]

Table 2
LC procedures used for analysis of local anesthetics.

Compound	Matrix	Extraction method	Stationary phase	Mobile phase	Detection mode	Quantitative analysis	Reference
Lidocaine	Human urine	SPME	C8	25 mM phosphate buffer pH 4.0 containing 15% acetonitrile 0.025% triethylamine	UV 210 nm	External standard	[5]
Bupivacaine	Cerebrospinal fluid	Dialysis	C18	Acetonitrile 230 ml and NaH2PO4 pH 2.1, 0.01 M 770 ml	UV 205 nm	Internal standard	[14]
Ropivacaine Bupivacaine	Human plasma	Direct injection	Precolumn Biotrap 500 C 18 C 18	1. 5% 2-propanol in phosphate buffer pH 7.4 2. 65% methanol in phosphate buffer pH 7.4	UV 240 nm	External standard	[15]
Lidocaine	Dog plasma	SPE	C18	Acetonitrile: pH 5.9 phosphate buffer (20:80, v/v)	UV 210 nm	Internal standard	[16]
Bupivacaine Mepivacaine Prilocaine	Human plasma	Dialysis	C18	Acetonitrile: 0.05 M phosphate buffer pH 6.0 (50:50, v/v)	UV 220 nm	External standard	[17]
Procaine Tetracaine	Human plasma	Direct injection	C18	Micellar mobile phase 0.15 M SDS, 0.5% triethylamine, 0.05 M phosphate buffer pH 2.5 and 10% propanol	UV 300 nm	External standard	[18]
Ropivacaine Bupivacaine	Human plasma	MIPs – SPE	YMC Basic	0.1% formic acid in acetonitrile: water (7:3)	MS/MSESI$^+$ triple quadrupole	Internal standard	[19]
Tetracaine Procaine Benoxinate Dibucaine Prilocaine Lidocaine Bupivacaine Mepivacaine	Human plasma and urine	LLE	-Lichrospher 100 Diol -Lichro CHART	Acetonitrile:water – 0.09% Formic acid and 20 mM amonium acetate 40:60	MS/MS ion trap SSI, APCI	Internal standard	[20]
Ropivacaine PPX 3-OH ropivacaine Lidocaine Bupivacaine	Human plasma	MEPS	C8	Methanol: water – 0.1% formic acid (1:1,v/v)	MS/MSESI$^+$ triple quadrupole	Internal standard	[21]
Prilocaine Lidocaine Ropivacaine Bupivacaine	Human plasma	SLM	C18	Methanol: 0.025 M phosphate buffer pH 2.5 (30:70, v/v)	UV 210 nm	External standard	[22]
Prilocaine Lidocaine Ropivacaine Bupivacaine	Human plasma	SLM	C18	Methanol: 0.025 M phosphate buffer pH 2.5 (30:70, v/v)	UV 210 nm	External standard	[23]
Bupivacaine Mepivacaine Prilocaine Ropivacaine	Human serum	LLE	Guard column C18, Synergy 4μ Polar C18 column	Acetonitrile: 2 mM ammonium acetate: formic acid Solvent A – 5:95:0.2 Solvent B – 95:5:0.2	MS/MSESI$^+$ triple quadrupole	External standard	[24]
Bupivacaine Mepivacaine Ropivacaine	Human serum	LLE	C18	Acetonitrile: methanol: 30 mM NaH2PO4 pH 5.6 (1:1:3)	UV 210 nm	Internal standard	[25]
Ropivacaine	Human plasma	Dialysis	C8	A: 0.05% trimethylamine in acetonitrile B: 2 mM ammonium formate buffer (pH 3.0)	MS ESI+	Internal standard	[26]
Lidocaine Bupivacaine Tetracaine	Human urine	LPME	C18	Acetonitrile and triethylamine aqueous solutions 11 mM: 0.1% phosphoric acid aqueous solutions (10:90, v/v)	UV 210 nm	External standard	[27]

Proparacaine	Aqueous humour sample	LLE	C8	Acetonitrile: NaH2PO4 20 mM, pH 3.0, (30:70, v/v)	UV 220 nm	Internal standard	[28]
Procaine P-amino-benzoic acid	Methanol/water solution	Direct injection	C18	A: 10 mM ammonium acetate pH 4.0 B: 0.1% formic acid in methanol Gradient elution	MS/MS ESI$^+$ triple quadrupole	Internal standard	[29]
Articaine	Water solution	LLE	Chiralcel OD ChirobioticV C18	n-hexane and 2-propanol 5% acetonitrile in triethylammonium acetate 7 mM pH 6.5	UV 274 nm MS EI	External standard	[30]
Articaine	Human plasma	LLE	C18	Acetonitrile: 0.1% formic acid in water (40:60, v/v)	MS/MS ESI$^+$ ion trap	Internal standard	[31]
Mexiletine	Human plasma	LLE + SPE	C18	Acetonitrile:water (80:20, v/v)	UV 458 nm	Internal standard	[32]
Lidocaine 2,6-xylidine MEGX GX	Human plasma and urine	Plasma-ultrafiltration Urine-SPE	YMC basic column Guard column C8	A: 0.1% formic acid in acetonitrile:water (0.5:99.5, v/v) B: 0.1% formic acid in acetonitrile:water (80:20, v/v) Gradient elution	MS/MS ESI$^+$ triple quadrupole	Internal standard	[33]
Lidocaine	Human plasma	LLE	C18 Guard column C18	Acetonitrile: 26 mM ammonium acetate pH 4.5 (70:30, v/v)	MS/MSAPCI triple quadrupole	Internal standard	[34]
Ropivacaine Bupivacaine	Human plasma	Dialysis + SPE	C18	Acetonitrile: 50 mM ammonium acetate buffer pH 7.0 (60:40, v/v)	MS ESI+	Internal standard	[35]
Ropivacaine PPX	Human plasma	Microdialysis	C18	A: methanol-ammonium formate pH 3.0 (5:95) B: methanol-ammonium formate pH 3.0 (50:50)	MS/MS ESI$^+$ triple quadrupole	Internal standard	[36]
Bupivacaine	Human plasma	LLE	C18	Acetonitrile: 0.1% formic acid in water (50:50, v/v)	MS/MS ESI$^+$ ion trap	Internal standard	[37]
Tricaine	Fish tissue	LLE + SPE	C18	Methanol:water:acetic acid (65:35:1)	MS/MS ESI$^+$ triple quadrupole	Internal standard	[38]
Articaine Articainic acid	Human serum	SPE	C8	880 ml potassium dihydrogenphosphate (0.02 mol/l 0.5 ml phosphoric acid, pH 3) and 120 ml acetonitrile	UV 274 nm	External standard	[39]
Procaine Lidocaine	Human saliva	LLE	C18	Methanol:water:formic acid (80:20:0.1, v/v/v)	MS/MS ESI$^+$ triple quadrupole	External standard	[40]
Sufentanil	Human plasma	SPE	HILIC	A: water/formic acid/1 M ammonium acetate (100:0.25:0.5, v/v/v, pH 2.8) B: acetonitrile/formic acid/1 M ammonium acetate (100:0.25:0.5, v/v/v)	MS/MS ESI$^+$ triple quadrupole	Internal standard	[41]
Lidocaine	Human blood Cerebrospinal fluid	LLE	C18	Methanol: diethylamine-acetic acid buffer pH 4 (22:78, v/v)	UV 263 nm	Internal standard	[42]
Lidocaine	Human plasma	SPE	C8	Acetonitrile: 10 mM ammonium acetate (pH 3.8) with gradient	MS/MS ESI$^+$ triple quadrupole	Internal standard	[43]
Lidocaine MEGX GX	Animal plasma	LLE	C18	Acetonitrile: 0.01 M ammonium acetate (60:40, v/v)	MS/MS ESI$^+$ ion trap	Internal standard	[44]
Dibucaine	Human serum	SPE	C18	Acetonitrile: 10 mM ammonium formate with gradient	MS ESI+	Internal standard	[45]
Procaine Lidocaine Ropivacaine Tetracaine Bupivacaine	Human plasma	LLE	C18	Acetonitrile: 30 mM potassium dihydrogen phosphate bufferwith 0.16% triethylamine, pH adjusted to 4.9 with phosphoric acid (37:63, v/v)	UV 210 and 290 nm	Internal standard	[46]
Ropivacaine	Human serum Human drainage blood	LLE	Zorbax SB-Aq	Acetonitrile: 4% 2-propanol in 10 mM ammonium acetate to pH 6.5 with formic acid with gradient	MS/MS ESI$^+$ ion trap	Internal standard	[47]
Mepivacaine Lidocaine	Equine plasma	On-line SPE	C18	Methanol 0.1% methylamine: 0.1% ammonium acetate Gradient elution	MS/MS ESI$^+$ triple quadrupole	Internal standard	[48]

have been tested. The effects of adsorption and desorption time, sample pH, temperature and salt additions were studied.

Polydimethylsiloxane fibre, pH 9.5, 0.3 g/ml NaCl, 45 min extraction time and 60 °C extraction temperature represented the optimal conditions for the determination of lidocaine in human urine [5].

Two aspects are worth emphasizing with non-headspace SPME: (a) since the sample is in direct contact with the fibre, strong acidic or alkaline conditions must be avoided and, (b) for whole blood, plasma or serum samples, deproteinisation is recommended because proteins may be also adsorbed.

2.4. Membrane-based sample preparation

2.4.1. Dialysis

Dialysis is the most widely used membrane-based sample preparation for determination of free and total local anesthetics concentrations in biological fluids [14,17,26,35,36]. In on-line combinations was mainly used with LC [17,36].

A method combining dialysis and enrichment of the dialysate on pre-column packed with cation-exchange material was developed for LC determination of prilocaine, mepivacaine and bupivacaine (total concentration) in human plasma [17]. An automated instrument, ASTED was utilised for this purpose. At plasma sample, spiked with anesthetics, monochloroacetic solution was added for protein-releasing.

Free concentration of ropivacaine, ropivacaine and bupivacaine, ropivacaine and PPX in plasma samples using equilibrium-dialysis followed by LC-MS was reported in several works [26,35,36]. Prior to dialysis plasma sample, spiked with drugs, and buffer was incubated at 37 °C for 15 min–1 h to assure protein binding. The sample and buffer (0.2 M potassium phosphate buffer pH 7.4) were separated by a cellulose membrane. The dialysate was directly injected into LC-MS system [26] or was processed by SPE using Isolute SCX cartridge [35].

2.4.2. Supported liquid membrane extraction

Supported liquid membrane (SLM) extraction consists of two processes: extraction of analyte from the donor phase into an organic solvent found in the pores of a membrane and back-extraction from the organic phase into an aqueous acceptor phase. The extraction is achieved by careful choice of the pH on either side of the membrane to control the change of the analytes. In determination of basic drug (total concentration) the donor phase pH should be two units higher than p*Ka* and the acceptor phase pH should be two units smaller than p*Ka*. SLM extraction was utilised for the determination of drug–protein binding and free drug concentration in plasma [22,23]. Local anesthetics studied in these works were: prilocaine, lidocaine, ropivacaine and bupivacaine. An automatic sample processor ASPEC was employed for the on-line extraction. The donor phase (drug solution in buffer pH 7.35 or plasma) was pumped through the donor channel at constant flow rate. The acceptor phase was stagnant during the extraction process. The pH of acceptor phase was set between 4 and 7 [22] or 7.4 [23].

The drug–protein binding depends on many factors such as: physicochemical nature of the drug, the affinity between drug and protein, the drug and protein concentrations and the presence of other compounds (drugs or endogenous compounds).

There has been no consent reached on the nature of drug–protein binding.

Kakinohana and Okuda [52] considered that local anesthetic–protein binding is hydrophobic while Taheri et al. [53] reported it was hydrophobic and electrostatic, and concluded that hydrophobic binding was much stronger than ionic binding.

At pH 7.53 of the donor phase [22,23] prilocaine (p*Ka* 8) is found 74.7% in ionised state and 25.3% in non-ionised state (according to Henderson–Hasselbalch equation). Only in non-ionised state prilocaine can be extracted in the organic solvent (di-hexyl ether with 5% tri-octylphosphine oxide). If the binding protein–prilocaine is hydrophobic, the non-ionised state is bound to the protein and the ionised form cannot be extracted in the organic solvent, and subsequently, in the acceptor phase. So, it is quite unlikely to have a percentage of 55% protein binding for prilocaine [22].

3. Chromatographic separation

3.1. GC separation

GC separation of local anesthetics was performed with polar and non-polar stationary phases (Table 1). The selectivity of these stationary phases and the high capillary column efficiency makes the GC separation of local anesthetics from biological samples to be easier than LC separations. The local anesthetics and their metabolites have been thermally stable at injector, column and detector temperatures.

The samples were injected in splitless mode. In order to avoid interference of the analytes with endogenous compounds, the oven temperature was programmed in different conditions.

3.2. LC separation

LC separation of local anesthetics from biological samples has been carried out mainly using C18- and C8-silica (Table 2).

The factors that determine the separation and analysis in LC, in the rank of their importance are: pH of mobile phase, solvent type, column type, % organic solvent, buffer concentration and type. The solute retention changes with pH only when the pH of the mobile phase is within ±1.5 units of the p*Ka* value of the solute. As a result, if mobile phase pH is to have an effect on separation selectivity, the pH must be within this interval. Nevertheless, in most cases mobile phase pH was acid (Table 2). At acid pH the basic compounds are fully ionised, but at some degree still retained on the lipophilic stationary phase. It is important that the vast majority of the molecules of solute be in the same form, ionised or non-ionised. Usually, the mobile phase consists in water: acetonitrile or methanol in different proportions. Mobile phase pH was adjusted with phosphate, ammonium acetate buffer, formic and acetic acid, to acidic or neutral conditions. Trimethylamine and triethylamine were used as "competing base" which interact strongly with free silanol groups still accessible to the analytes.

For enantioseparation of articaine hydrochloride two chiral stationary phases Chiralcel OD and Chirobiotic V columns have been tested [30]. Chiral separations on a Chiracel OD column were performed under normal phase condition with n-hexane – 2-propanol liquid phase in different proportion, with ethanol as organic modifier.

On Chirobiotic V, three different solvent-elution modes were investigated: "normal phase mode" with n-hexane and 2-propanol in different proportions; "new polar organic mode" with methanol, acid acetic and triethylamine; "reverse phase mode" with triethyl ammonium acetate buffer (7 mM; pH 6.5) and acetonitrile (95:5, v/v). The authors reached the conclusion that both columns were effective for the analysis of articaine and its metabolite.

Escuder-Gilabert et al. [18] applied micellar liquid chromatography (MLC) for determination of procaine and tetracaine from plasma sample. MLC is a reversed-phase liquid chromatographic mode with mobile phases containing a surfactant (ionic or non-ionic) above its critical micellar concentration. In MLC electrostatic, hydrophobic and steric interactions between the solute and both stationary and mobile phases occur. Both equilibria are affected by a variety of factors such as the nature and concentration of surfactant and additives (salt or organic modifier), temperature, ionic

Table 3
Ions used for GC–MS and LC-MS/MS analysis of local anesthetics.

Compound	Ion (*m*/*z*)	Precursor ion (*m*/*z*)	Product ion (*m*/*z*)	Reference
Ropivacaine	126 (EI)275 [M+H]+ ; 126 (CI)	275 $[M+H]^+$	126; 84; 56 (ESI)	[8,19,21,24,36,37,47]
Bupivacaine	140 (EI)	289 $[M+H]^+$	126; 140 (ESI); 140 (APCI)	[4,9,10,19,21,24,34,37]
D7-ropivacaine	133 (EI) 282 $[M+H]^+$; 133 (CI)	282 $[M+H]^+$	133 (ESI)	[8,19,22]
Tetracaine	58 (EI)	265 $[M+H]^+$	176 (SSI)	[9,20]
Procaine	86 (EI)	237 $[M+H]^+$	118 (APCI); 100; 120; 164 (ESI; SSI)	[9,29,40]
Benoxinate		309 $[M+H]^+$	118 (APCI); 100; 192; 219 (SSI)	[20]
Prilocaine	86 (EI)	221 $[M+H]^+$	86 (APCI; ESI; SSI)	[9,10,20,24]
Lidocaine	86 (EI)	235 $[M+H]^+$	86; 57.9 (APCI; ESI; SSI)	[9,10,20,21,33,34,40,43,44,48]
Mepivacaine	98 (EI)	247 $[M+H]^+$	98 (APCI; SSI)	[9,10,20,24,48]
Dibucaine	86 (EI)	344 $[M+H]^+$	245; 257 (APCI)	[9,10,20,45]
3-OH-ropivacaine		291 $[M+H]^+$	126 (ESI)	[21]
PPX		233 $[M+H]^+$	84 (ESI)	[21,36]
p-aminobenzic acid		137.05 $[M+H]^+$	77.04; 94.07; 120.04 (ESI)	[29]
N-acetylprocainamide		278.19 $[M+H]^+$	205.1; 162.08 (ESI)	[29]
Articaine		285 $[M+H]^+$	168 (ESI)	[31]
2,6-xilidine		122 $[M+H]^+$	105 (ESI)	[33]
MEGX	58 (EI)	207 $[M+H]^+$	58 (ESI)	[9,33,44]
GX		179 $[M+H]^+$	122 (ESI)	[33,44]
Tricaine		166 $[M+H]^+$	94; 120; 138 (ESI)	[38]
Pentycaine	154 (EI)			[4]
Sufentanil		387 $[M+H]^+$	238	[41]

strength, and pH. In the above-mentioned work [18], the mobile phase was prepared from aqueous solution of 0.15 M sodium dodecylsulfate, 0.5% triethylamine, and 10% 1-propanol. Mobile phase pH was adjusted to 2.5 with phosphate buffer.

An alternative to conventional reversed-phase LC or normal phase LC for the analysis of biological sample is the hydrophilic interaction liquid chromatography (HILIC). This method is often used for the separation of very polar molecules being able to solve many problematic retention and separation problems. The mobile phase is composed of a high percentage of organic solvent (e.g. acetonitrile) and a small percentage of water with volatile buffer. The main advantages of HILIC include high selectivity and sensitivity, shorter analysis time, more symmetrical peaks. The volatility of the mobile phase due to high content of acetonitrile or methanol makes the coupling with electrospray mass spectrometry to be simpler. For the separation of sufentanil in human plasma Schmidt et al. [41] used an Alltima HP HILIC column. Another stationary phase designed to retain hydrophilic compounds is Zorbax SB-Aq. Ropivacaine in serum, ultrafiltrate and drainage blood, free and total concentrations, was separated on this column [47].

4. Detection

4.1. GC detection

As can be seen in Table 1, the detection in GC analysis was performed by flame ionisation detectors (FID), nitrogen-phosphorus detectors (NPD) and mass spectrometer (MS). From the point of view of quantification limit (LOQ) these detectors are comparable (ng/ml).

The ions used for quantification and confirmation purposes by MS are summarised in Table 3. Electron impact ionisation (EI) was utilised in most of the cases but Engman et al. [8] used chemical ionisation (CI) with NH_3.

The fragment ion of bupivacaine observed at *m*/*z* 140 corresponds to the butane-1-piperidinyl group, the fragment ion of mepivacaine at *m*/*z* 98 corresponds to the methane-1-piperidinyl group, the fragment ion of lidocaine, procaine, dibucaine and prilocaine at *m*/*z* 86 corresponds to ethylpropylamine, the fragment ion of ropivacaine, 3-OH-ropivacaine, 4-OH-ropivacaine at *m*/*z* 126 corresponds to the propane-1-piperidinyl group and the fragment ion of pentycaine at *m*/*z* 154 corresponds to the pentyl-1-piperidinyl group.

4.2. LC detection

Detection in LC analysis was performed by UV, MS, MS/MS detectors (Table 2).

The LC-MS and LC-MS/MS analysis of local anesthetics was carried with electrospray ionisation (ESI), atmospheric-pressure chemical ionisation (APCI), sonic spray ionisation (SSI). Selected ion monitoring (SIM), full scan and multiple reaction monitoring (MRM) were used for the quantitation of local anesthetics. Operation of mass spectrometry in SIM mode gives enhanced sensitivity, while operation in MRM mode enables the identification of the drug. All SSI, APCI and ESI spectra gave base peaks of protonated molecular ions. As regards product ions, their formation depends on collision energy [29], the way the ionisation is performed, and on compound structure.

Fragmentation of amine containing side chains was investigated by ESI-MS^n and EI-MS [40]. Procaine has a carbon chain ending with a tertiary amine function and two methylene groups [-COO-CH_2-CH_2-N(C_2H_5)$_2$]. ESI-MS^n fragmentation gives rise, beside other fragments, to diethylamine HN(C_2H_5)$_2$ (*m*/*z* 73), because of the cleavage of C–N bond. In EI-MS, procaine is fragmented to give charged N-containing species such as $CH_2=N^+(C_2H_5)_2$ at *m*/*z* 86. Lidocaine has a completely different behaviour in ESI-MS^n. It has a carbon chain with an amide group and only one methylene group attached to a xylene ring [-NH-CO-CH_2-N(C_2H_5)$_2$]. For ESI-MS^n, it would appear that at least two methylene groups are required adjacent to the end N atom for cleavage of C–N bond. The fragmentation of lidocaine (both ESI-MS^n and EI-MS) gives rise to a signal at *m*/*z* 86.4 corresponding to $CH_2=N^+(C_2H_5)_2$ and *m*/*z* 58.1 corresponding to $CH_2=N^+H(C_2H_5)$. The cleavage of C–N bond does not occur in this case. The authors suppose this is probably due to the amide bond in lidocaine being stabilised by virtue of resonance with the xylene ring.

Precursor ions and their product ions used for quantification and confirmation purposes are summarised in Table 3.

4.3. Application of the methods to biological samples

The pharmacokinetics of local anesthetics has been studied in epidural, intravenous regional topical and mandibular nerve block anesthesia. Besides the method of administration and the administered quantity, the blood level of local anesthetics is influenced by the following factors: rate of which the drug is absorbed into

cardiovascular system, rate of distribution of the drug from the vascular compartment to the tissue (which is more rapid in healthy patients), elimination of the drug trough metabolic and/or excretory pathways.

Biotransformation of local anesthetics is important from toxicity point of view. The primary site of biotransformation of anesthetic amide group is the liver. Patients with lower than normal hepatic blood flow (hypotension, congestive heart failure) or poor liver function (cirrhosis) are unable to biotransform amide local anesthetics at a normal rate. This slower than normal biotransformation rate leads to increased anesthetic blood levels and potentially increased toxicity. Amino-ester anesthetics are metabolised in the blood and only 5–10% in liver [55]. The metabolites can be inert or toxic. The half-life is larger in amide group (90 min for lidocaine) against ester-type (20 min for articaine) [56].

Most of the papers reported a pharmacokinetic study after the administration of local anesthetics in therapeutic doses. Tahraoui et al. [4] presented a comparison of results obtained by LC and GC–MS for the determination of bupivacaine in plasma from a patient treated with solution of bupivacaine (20 ml of a 0.75% w/v) injection. The blood was collected in heparinized tubes at 0, 15, 30 and 60 min and 3, 8, 24 h after administration of drug. The maximum concentration occurred 15 min after administration (42.4 ± 0.95 ng/ml for LC and 42.56 ± 0.89 ng/ml for GC–MS). The authors reached the conclusion that the GC–MS procedure was more sensitive, due to its higher specificity, allowing the analysis of smaller volumes of plasma than required for LC.

The method presented by Laroche et al. [7] has used to measure MEGX (metabolite of lidocaine) after intravenous injection of 1 mg/kg of lidocaine in order to evaluate liver function. The blood samples were collected from two subjects, one with normal hepatic function and the other with cirrhosis of the liver, from 15 min to 24 h after injection. The authors' conclusion was that MEGX formation and elimination in subjects with cirrhosis are slower than in normal subjects. Thus, the determination of MEGX production can be a useful test to distinguish cirrhotic subjects from normal ones.

The total (and free) maximum concentration of bupivacaine and ropivacaine in plasma from neonates after epidural anesthesia (simple injection of 1 ml/kg of venous blood) have been 647 (1.9) ng/ml and 636 (2.0) ng/ml at 45 min for bupivacaine and 855 (3.3) ng/ml for ropivacaine at 60 min [35]. A similar concentration (0.6 μg/ml) was noticed after epidural administration of a loading dose of 1.25 mg/kg, followed 60 min later by an infusion of 0.25 mg/kg/h over 71 h in three paediatric patients.

Mepivacaine in human serum collected from three healthy volunteers, following the injection of 1.7 ml mepivacaine 3% in a dental clinic at 5, 15, 30, 45, and 60 min after administration concluded that the highest concentration of mepivacaine (0.8–1 μg/ml) occurred between 15 and 30 min after the injection [12].

Popescu et al. [54] studied the effect of propranolol pretreatment on mepivacaine serum concentration in dental patients. The study was made on 10 patients who ingested 30 mg propranolol 2 h before administration of 51 mg mepivacaine (single dose) for posterior superior alveolar nerve block. Mepivacaine in venous serum was measured after 5, 15, 30, 45 and 60 min from injection. The concentration of mepivacaine was very significantly increased by propranolol. This could be explained by the mechanisms of action of propranolol in liver: decreasing hepatic blood flow or inhibiting the enzymes responsible for metabolising the local anesthetic.

Richter and Oertel [39] described a method for the determination of articaine and its metabolite, articainic acid in human serum after an oral submucosal injection of 16 mg articaine hydrochloride. The sensitivity of the method is sufficient for the determination of the analytes in human serum, even in this case of low dose administration. The highest concentration was 125 ng/ml, obtained at 12 min after the injection moment.

Smith et al. [41] developed a method for the determination of S-ropivacaine and sufentanil in parturients and neonates' plasma, following patient-controlled epidural analgesia. The LC-MS/MS method is sensitive enough to detect the low concentrations (<10 pg/ml) in parturient plasma and in the arterial umbilical plasma of their neonates.

Qin et al. [46] described a HPLC-UV method for the simultaneous determination of procaine, lidocaine, ropivacaine, tetracaine and bupivacaine in human plasma. They used neostigmine methylsulfate as anticholinesterase. The method proved to be applicable to a preliminary pharmacokinetic study in which 10 adult patients were administered a mixture of local anesthetics (2 mg/kg each) during brachial plexus block.

The therapeutic concentrations in blood plasma of several local anesthetics are: lidocaine and mepivacaine 2–5 μg/ml, bupivacaine 1.7–4 μg/ml, procaine 21–86 μg/ml [1]. Over passing these limits can cause toxic symptoms, such as mental derangement, vertigo, anxiety, delirium, paresthesia, hypotension, CNS suppression and convulsion or even fatality. A few papers monitored these high concentrations of local anesthetics.

Watanabe et al. [10] performed a toxicological analysis of mepivacaine and lidocaine in human blood obtained from a suspected victim of local anesthetics poisoning. The concentrations of the left and right heart blood were 18.6 and 15.8 μg/g for mepivacaine, 0.14 and 0.17 μg/g for lidocaine, respectively. The concentration of mepivacaine in blood was higher than the therapeutic level.

Another RP-HPLC method with UV detection was performed by Chen et al. [42] and it was applied in the forensic toxicology, for the determination of lidocaine in human blood and cerebrospinal fluid in 10 cases. The blood concentration of the analyte was higher in the cerebrospinal fluid (20.3–185.6 μg/ml) than in plasma (0.68–34.4 μg/ml).

Saito et al. [45] investigated dibucaine concentrations in a poisoned patient serum. The concentrations were determined to be 675.7 and 218.6 μg/ml respectively 3 and 9 h after injection.

Other authors performed analysis on animals. Leung et al. [43] reported a method for the determination of 75 basic drugs in equine plasma by LC-MS/MS. The method provided a fast analysis time (8 min including post-run and equilibration time). The LODs were 0.5 and 1.25 pg/ml for lidocaine and cocaine respectively. Lidocaine and its metabolites MEGX and GX were analysed by LC-MS/MS [44]. The method showed good linearity, low LODs and LOQs. It was successfully applied in pharmacokinetic studies after application of a transdermal patch in dogs and an intravenous infusion in horses. Screening of drugs in equine plasma by LC-MS/MS was performed by Kwok et al. [48]. Among other compounds, mepivacaine and lidocaine were determined in pre-race plasma samples from racehorses with LODs of 19 and 75 pg/ml respectively.

5. Conclusions and trends

This review illustrates the role of GC and LC in the analysis of local anesthetics and their metabolites for studies of pharmacology and toxicology. In general, a reliable analytical method should fulfil the validation requirements that include precision, accuracy, selectivity, sensitivity, reproducibility and stability. These targets depend on sample preparation, chromatographic separation and detection. The complexity of the matrix (whole blood, plasma, serum, urine, tissue) makes sample preparation a very important step in analysis. Some general conclusion and trends regarding

analysis of local anesthetics and their metabolites in biological samples are presented below:

a) Alkalinisation of the sample (blood, urine, serum or plasma) is necessary before liquid–liquid extraction with an organic solvent, as well as before solid-phase-extraction with a lipophilic sorbent.
b) The ester-type local anesthetics are easily hydrolysed by the action of pseudocholinesterase in blood. In order to inhibit activity of pseudocholinesterase, before acquiring the blood sample in heparinized tube, a neostigmine aqueous solution (or another inhibitor) must be introduced in the tube.
c) The analysis of the metabolites (glucuronides or sulphates) can be performed only after acidic or enzymatic hydrolysis.
d) LLE and SPE are widely used for extraction of local anesthetics from biological samples. Good extraction recoveries are obtained with these methods. Trends in sample preparation are to reduce the use of solvents, to simplify manipulations and to reduce the time necessary for sample preparation. Miniaturisation, high-throughput systems, and automation are of interest. Based on principles of extraction techniques, new automated and on-line coupled with analytical instruments methods have been developed. Automation improves precision and accuracy and reduces risk of sample contamination. SPE and membrane-based techniques are most attractive for on-line coupling.
e) In GC, local anesthetics are successfully separated on capillary columns with polar or non-polar stationary phases.
f) Reversed-phased columns C18 or C8-silica are widely used for the analysis of local anesthetics. In classic LC the mobile phase has an essential role in selectivity. The present trends in LC consist in finding selective stationary phases (HILIC and Zorbax SB-Aq) and efficiency optimisation using sub-2-μm particle packed columns and high temperatures.
g) For detection, FID and MS (GC) and triple quadrupole or ion trap MS/MS (LC) detectors were used. Using LC-MS/MS methods, local anesthetics and especially their metabolites are easily identified and LODs are slightly higher than those obtained in GC-FID and GC–MS.

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